

## EFFECT OF VITAMIN DEFICIENCIES ON CARBOHY- DRATE METABOLISM.

### I. HYPOGLYCEMIA ASSOCIATED WITH ANHYDREMIA AND DIS- TURBANCE IN HEMATOPOIETIC FUNCTION IN NURSING YOUNG OF THE ALBINO RAT SUFFERING FROM UNCOMPLICATED VITAMIN B DEFICIENCY.\*

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The purpose of this investigation was to study the concentration of blood sugar in nursing young of the albino rat suffering from uncomplicated vitamin B deficiency in the state of prolonged maintenance. The details of biological technique for the production of such avitaminosis will appear elsewhere, but essentially it consists of the administration to the lactating mothers of a diet, containing an abundance of vitamin F<sup>1</sup> in the form of autoclaved yeast, but deficient in vitamin B. Small daily allowances of dehydrated yeast during the early period of lactation, together with the storage of vitamin B from the previous diet, insure prolonged maintenance but no growth in the nursing young during the later periods of lactation. The nurslings eventually develop posterior paralysis, labored respiration, cyanosis, and finally death occurs, unless vitamin therapy is instituted.

In this investigation we have studied forty-four pathological nursing young suffering from uncomplicated vitamin B deficiency and six nurslings suffering from a deficiency of vitamins B and F (vitamin B complex). As controls we have studied forty animals on Ration 1145 (containing an abundance of vitamins B and F)

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<sup>1</sup> The term "vitamin F" refers to the stable antipellagic factor, according to a nomenclature suggested in a previous communication (1).

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(2), and six young on Stock Diet 1 (3). In our preliminary work we have found the same degree of variations among individual animals on the two types of diets; therefore, we have taken most of our control determinations on rats receiving Ration 1145, such animals having been at that time available for this work. The blood sugar concentration was studied in all the 50 pathological animals. In thirty-nine of these we have in addition followed the changes in the concentration of hemoglobin, erythrocytes,<sup>2</sup> and

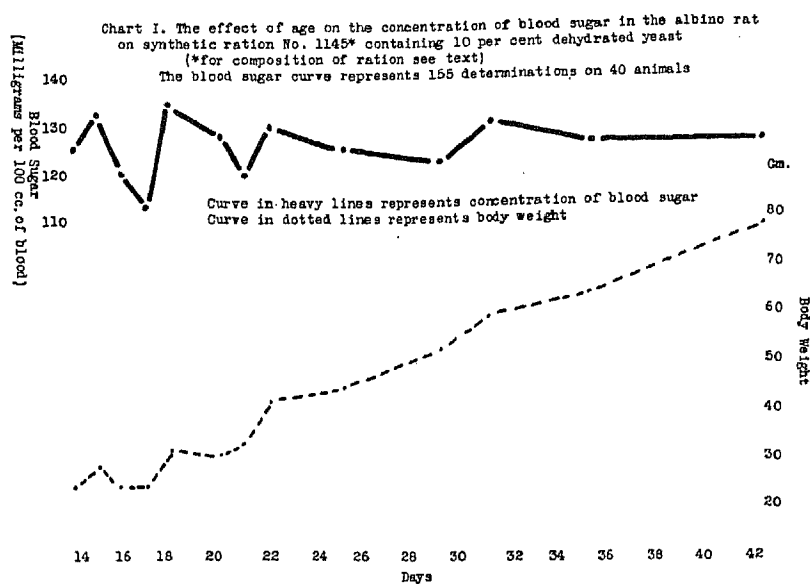


CHART I.

serum proteins. All animals were bled peripherally. After a number of preliminary trials we found that there were less variations in determinations of blood constituents, since as little as 0.05 cc. of blood had to be employed for micro determinations, if we used non-oxalated blood. For the determination of all the above mentioned blood constituents we used 0.31 cc. of blood. The Kramer-Gittelman micro method for blood sugar (4) was found most suitable for our purpose, since only 0.05 cc. of blood

<sup>2</sup> Credit is due Miss D. J. Walker for making most of the erythrocyte counts.

is necessary, and the determinations checked with the gasometric method of Van Slyke and Hawkins (5) to the extent of  $\pm 1$  per cent.<sup>3</sup> The rest of the blood constituents were determined according to technique described in the preceding paper (2).

Chart I shows the effect of age on the concentration of blood sugar on synthetic Ration 1145, abundant in vitamins B and F. It will be noted that between the 14th and 22nd day of lactation the concentration of blood sugar was between 113 and 135 mg. per cent. Between the 22nd and 42nd day, representing a post weaning period, the blood sugar maintained almost a plateau, fluctuating from 120 to 130 mg. per cent. On Stock Diet 1 the blood sugar between the 18th and 38th day varied from 110 to 138 mg. per cent. On neither of these two types of diets did the concentration drop below 110 mg. per cent. With these figures as a basis of comparison, all of our pathological animals showed marked hypoglycemia during the vitamin B depletion period long before any losses of body weight had taken place.

From the standpoint of concentration of blood sugar, the pathological animals fall into two groups, one that showed progressive hypoglycemia, and the other which, during the early period of vitamin B depletion, had a hypoglycemia of 10 to 20 per cent, followed by a precipitous drop of 40 to 50 per cent during the pre-mortal state. All the nurslings suffering from a deficiency of the vitamin B complex showed the progressive type of hypoglycemia, while only seventeen young out of forty-four young in the prolonged maintenance state of uncomplicated vitamin B deficiency manifested that state of hypoglycemia.

Space does not permit us to show all of our results in detail. We are, therefore, submitting two illustrations, in Charts II and III, which are representative of the two types of hypoglycemia encountered. In one case we are also showing the effect of vitamin B therapy on growth, concentration of blood sugar, hemoglobin, erythrocytes, and serum proteins. Seven additional vitamin therapy cases were studied.

*Chart II. Nursling S-195.*—The progressive development of accentuated hypoglycemia is very striking in this nursling. The blood sugar was reduced in 10 days from 103 to 55 mg. per cent.

<sup>3</sup> We used a micro method, kindly furnished us by Dr. Van Slyke.

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The anemia and anhydremia are also quite evident in this animal. The anemia was evidenced not only by the precipitous drop in hemoglobin, during the last 4 days of the experiment, but also in

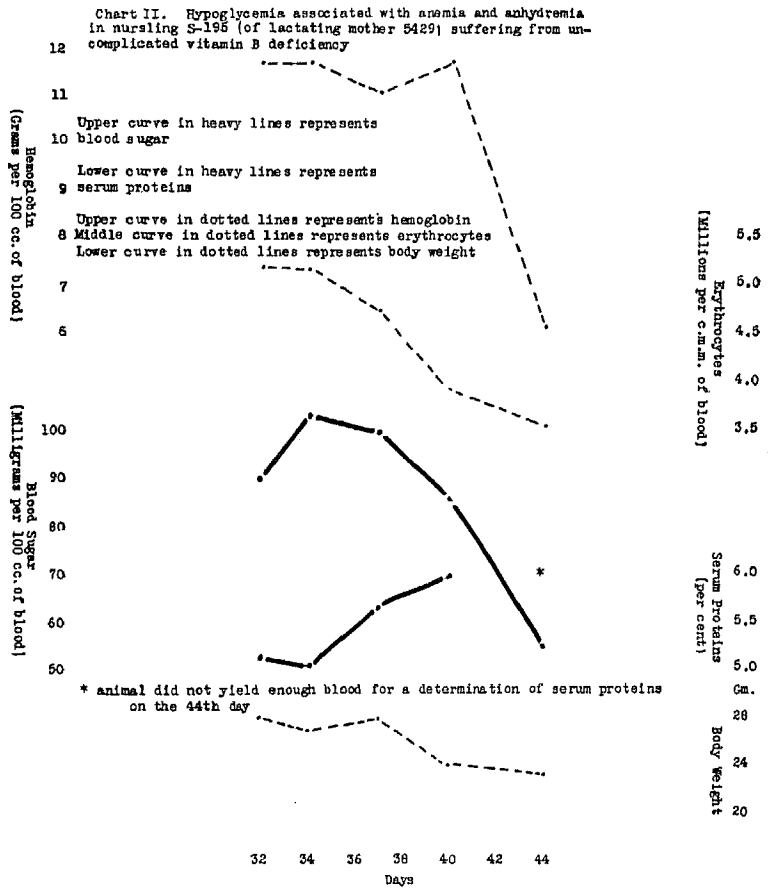


CHART II.

the continuous reduction in the concentration of the erythrocytes. Between the 32nd and 40th day there was an increase of 20 per cent in the concentration of serum proteins, although there was a loss of body weight to the extent of 14 per cent during the same interval, *i.e.* anhydremia.

Chart III. Nursing S-185.—The biochemical changes in this nursing preceding vitamin B therapy are of special interest. During the period the hemoglobin was falling (35th to 45th day) the

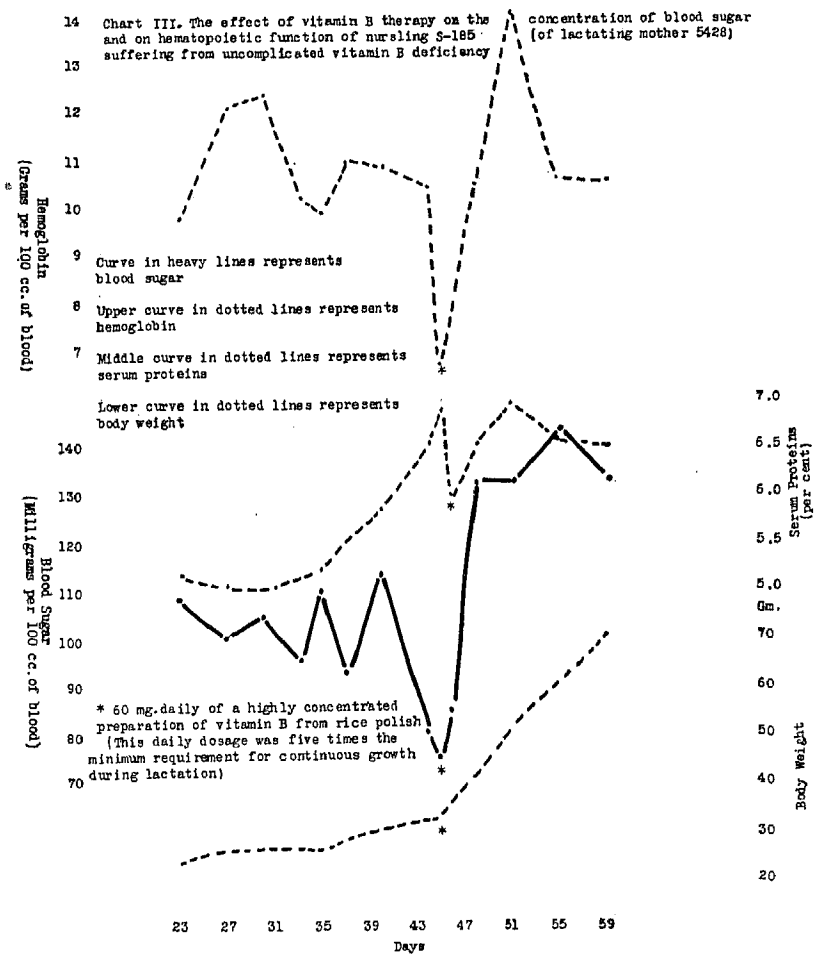


CHART III.

concentration of serum proteins was appreciably increasing. Such findings we interpreted to signify that there might be water lost from the blood serum and at the same time an anemia produced

in the individual. We have observed in a number of control animals that the concentration of serum proteins and hemoglobin does not necessarily occur in definite proportions. For instance, we found in several animals on control Ration 1145 the concentration of serum proteins to be 4.5 per cent and the hemoglobin concentration in the same individuals that were making excellent growth to vary from 7 to 12 gm. per 100 cc. of blood.

At the point of the precipitous drop of blood sugar and hemoglobin this nursling showed advancing symptoms of polyneuritis, and vitamin therapy was then instituted. The daily administration of 60 mg. of a highly concentrated extract of vitamin B from rice polishings<sup>4</sup> was initiated. The effect of vitamin B therapy was most pronounced on the concentration of blood sugar. In 3 days there was an increase of 77 per cent and the higher concentration was maintained throughout the rest of the experimental period. The influence of vitamin B administration on the regeneration of hemoglobin was equally marked. During the vitamin B depletion period the erythrocytes fell from 5.12 to 4.03 millions per c.mm. and on the 59th day when the experiment was terminated the concentration of red blood corpuscles was 6.80 millions per c.mm. (not shown on chart). The effect of vitamin B therapy on growth was also quite pronounced.

#### DISCUSSION.

While the character of the hematopoietic disturbance was different in each of the nursing young suffering from uncomplicated vitamin B deficiency, as evidenced by concentration of hemoglobin, erythrocytes, and serum proteins, the pathological symptom common to all of such nurslings was hypoglycemia. That the hypoglycemia was frequently progressive, and not just a premortal state, was apparent in seventeen cases out of forty-four studied. Since the hypoglycemia was quite evident during a period of prolonged maintenance produced by vitamin B depletion, so that the condition was not complicated by appreciable changes in body weight, there can be no question, then, that vitamin B had a determining influence on carbohydrate metabolism. Our results

<sup>4</sup> Prepared by one of us (B. S.) by a chemical procedure to be described later.

of hypoglycemia in polyneuritic nursing young of the albino rat are in agreement with the findings of Suzuki (6) of hypoglycemia in infantile beriberi. The hyperglycemia reported in avian beriberi (7) and in full grown rats suffering from a deficiency of the vitamin B complex (8) are not comparable with our work on nursing baby rats, and no attempt has, therefore, been made to review critically that literature. We are continuing our studies on animals during the post weaning period and also on adult rats.

The frequent rapid increases in the concentration of serum proteins encountered during periods of prolonged maintenance, and particularly during intervals of loss of body weight, unquestionably establish the fact that in uncomplicated vitamin B deficiency of nursing young of the albino rat we have pronounced anhydremia.

The marked anemia observed, as evidenced by the reduction in the concentration of hemoglobin to the extent of 100 per cent, may be open to criticism. In order to secure enough blood for the determinations of all the constituents studied, we were obliged to draw as much as 0.31 cc. of blood from baby rats, some weighing as little as 20 gm. If we calculated the blood volume as 6 per cent (9) (in the absence of direct blood volume determinations) we had to remove, in some cases, 26 per cent of the total blood of the animal at each bleeding. Realizing this situation, we planned to bleed the nurslings not more than twice a week. On the other hand, the external symptoms frequently warranted more frequent bleedings, in order to secure additional information before the onset of the premortal state of the animal. But, granting that the anemia encountered in our pathological nurslings had been produced partly by removal of blood from the animals, these young must have been unable to regenerate their blood because of vitamin B deficiency, since we bled the nurslings that received vitamin B therapy just as frequently, and the result was rapid blood regeneration instead of a development of anemia.

A word of explanation is necessary as to the vitamin B dosage employed in eight therapy experiments. A biological assay of the concentrated preparation of vitamin B from rice polishings employed in this investigation on several hundred individuals by our quantitative biological method (10) revealed a potency of 1 gm. containing 80 vitamin B units (uncomplicated); *i.e.*, 12 mg.

were found sufficient to produce a growth of 10 gm. in a 30 gm. nursing young during a period of 7 to 10 days. In an attempt to save nursing young in the terminal stages of uncomplicated vitamin B deficiency even 3 times the 12 mg. dosage proved ineffectual. We, therefore, used 60 mg., or 5 times the minimum dosage with great success. To date we have succeeded in saving three individuals in the terminal stage of the avitaminosis (two of which were in marked opisthotonos) that were failing, gasping for breath, by proceeding with administrations of 60 mg. daily allowances of the concentrated extract of vitamin B from rice polishings.

Our food consumption records show that the lactating mothers, whose young developed the avitaminosis, consumed daily about 50 per cent less metabolizable food products than is necessary for the optimum development of nursing young during lactation. Our tentative hypothesis, then, is that the insufficient food intake for normal lactation results in less carbohydrates accessible to the nursing young for energy metabolism, and hence the hypoglycemia.

The biochemical changes in the blood of the nurslings that received vitamin B therapy might be attributed to growth produced by increase in food consumption, which in turn, was influenced by the physiological action of vitamin B. The increases in the concentration of blood sugar and the regeneration of hemoglobin following vitamin B therapy, however, proceeded at a much more rapid rate than the increase of body weight.

#### SUMMARY.

1. Nursing young of the albino rat suffering from uncomplicated vitamin B deficiency develop marked hypoglycemia at a stage of the avitaminosis before loss of body weight has occurred.
2. In such avitaminosis nursing young of the albino rat also develop anhydremia and marked disturbance in hematopoietic function.
3. Vitamin B therapy produces a rapid increase in concentration of blood sugar, and a regeneration of blood in polyneuritic nursing young of the albino rat.

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